10090440 .070902

#5

Figure 1

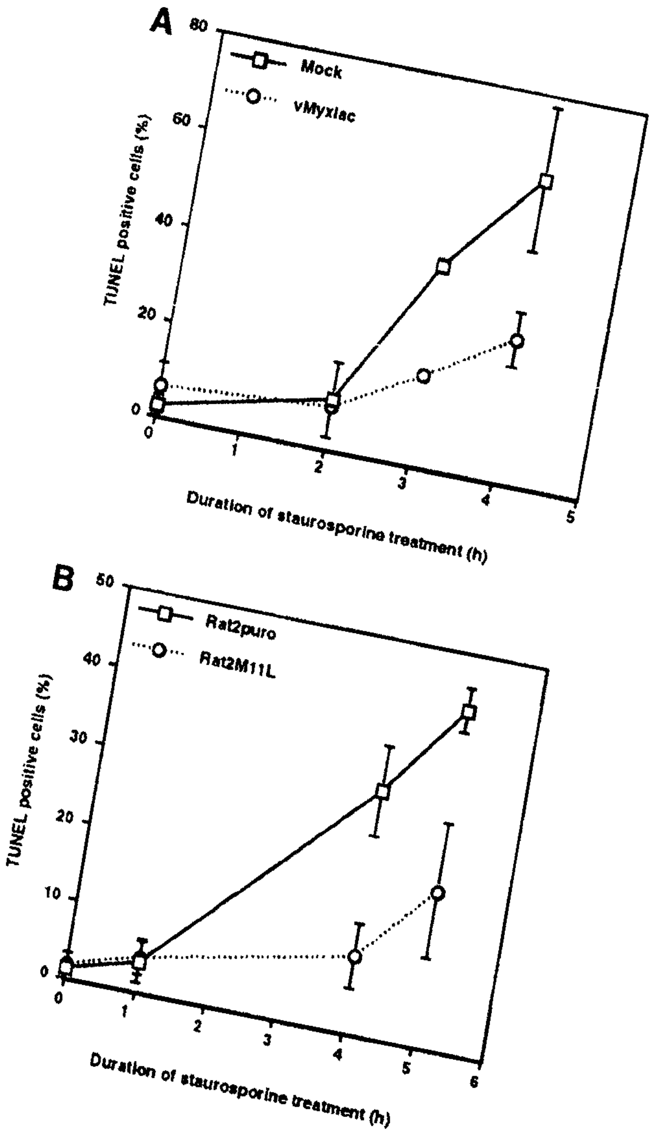
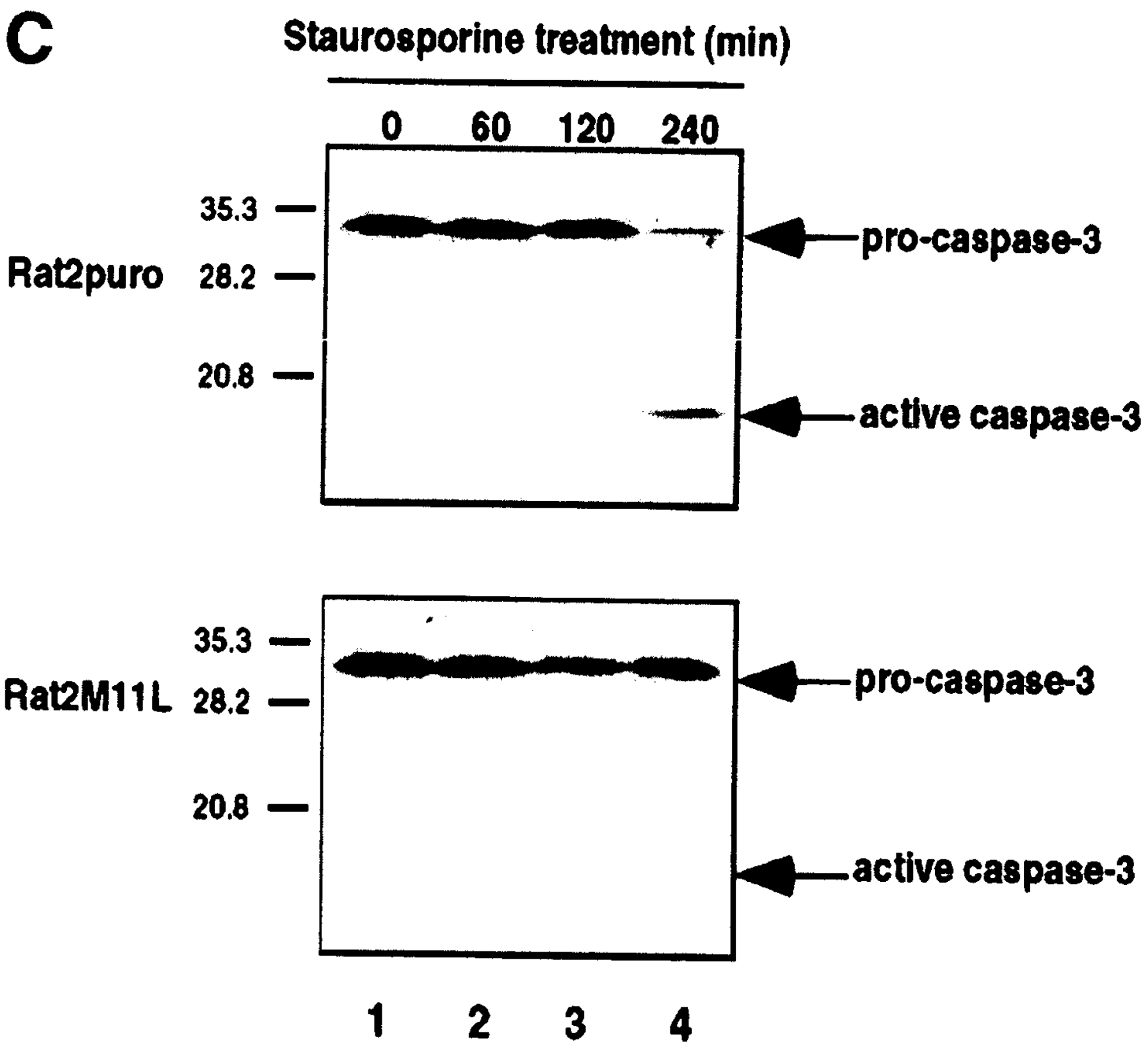


Figure 1 (con't)


C
Staurosporine treatment (min)
0
60
120
240
35.3
28.2
20.8
Rat2puro
pro-caspase-3
active caspase-3
35.3
28.2
20.8
Rat2M11L
pro-caspase-3
active caspase-3
1
2
3
4

10090440 .070902

Figure 2

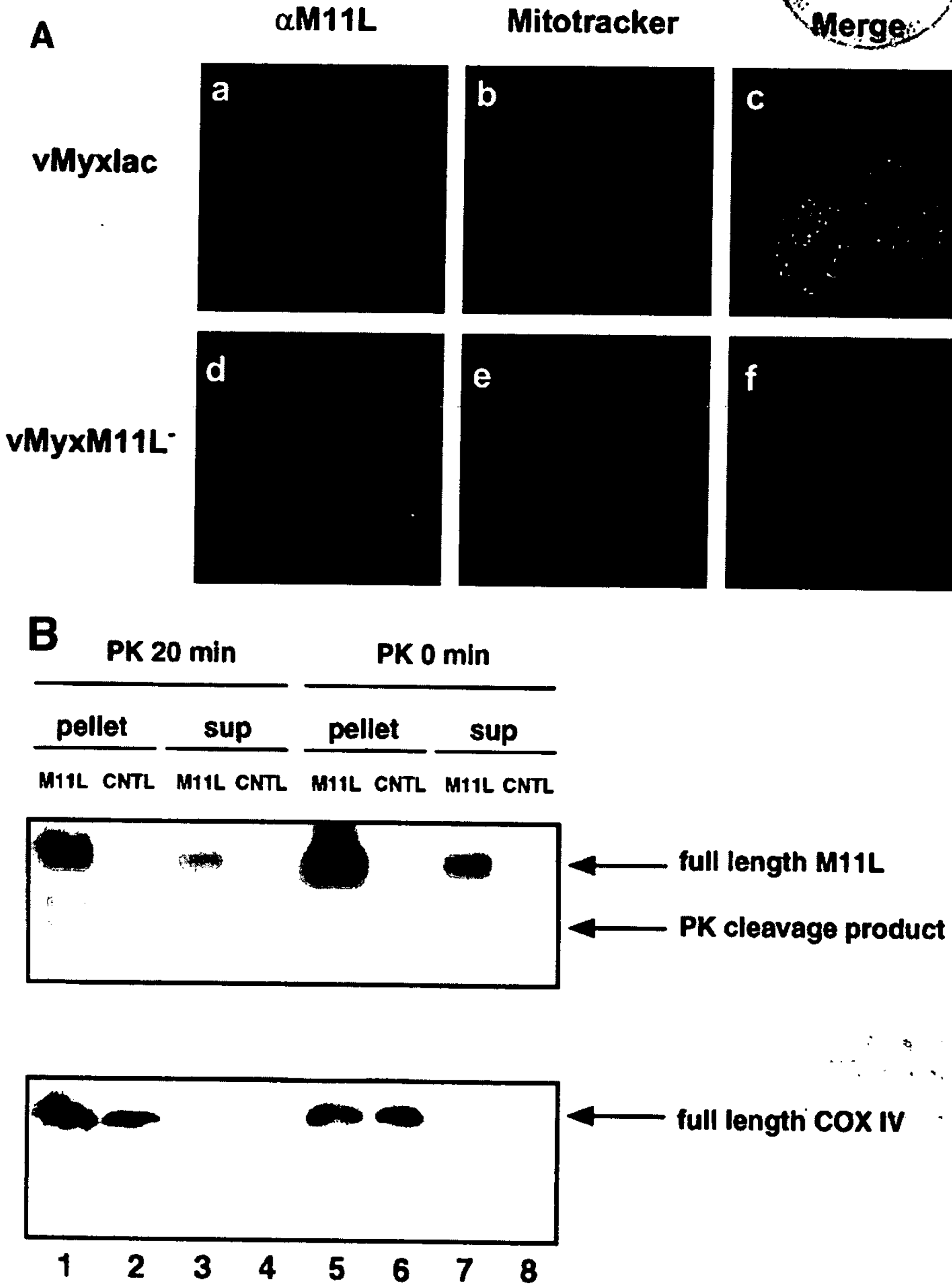

A
αM11L
Mitotracker
Merge
vMyxlac
vMyxM11L⁻
a
b
c
d
e
f
B
PK 20 min
PK 0 min
pellet
sup
pellet
sup
M11L
CNTL
M11L
CNTL
M11L
CNTL
M11L
CNTL
full length M11L
PK cleavage product
full length COX IV
1
2
3
4
5
6
7
8

Figure 3

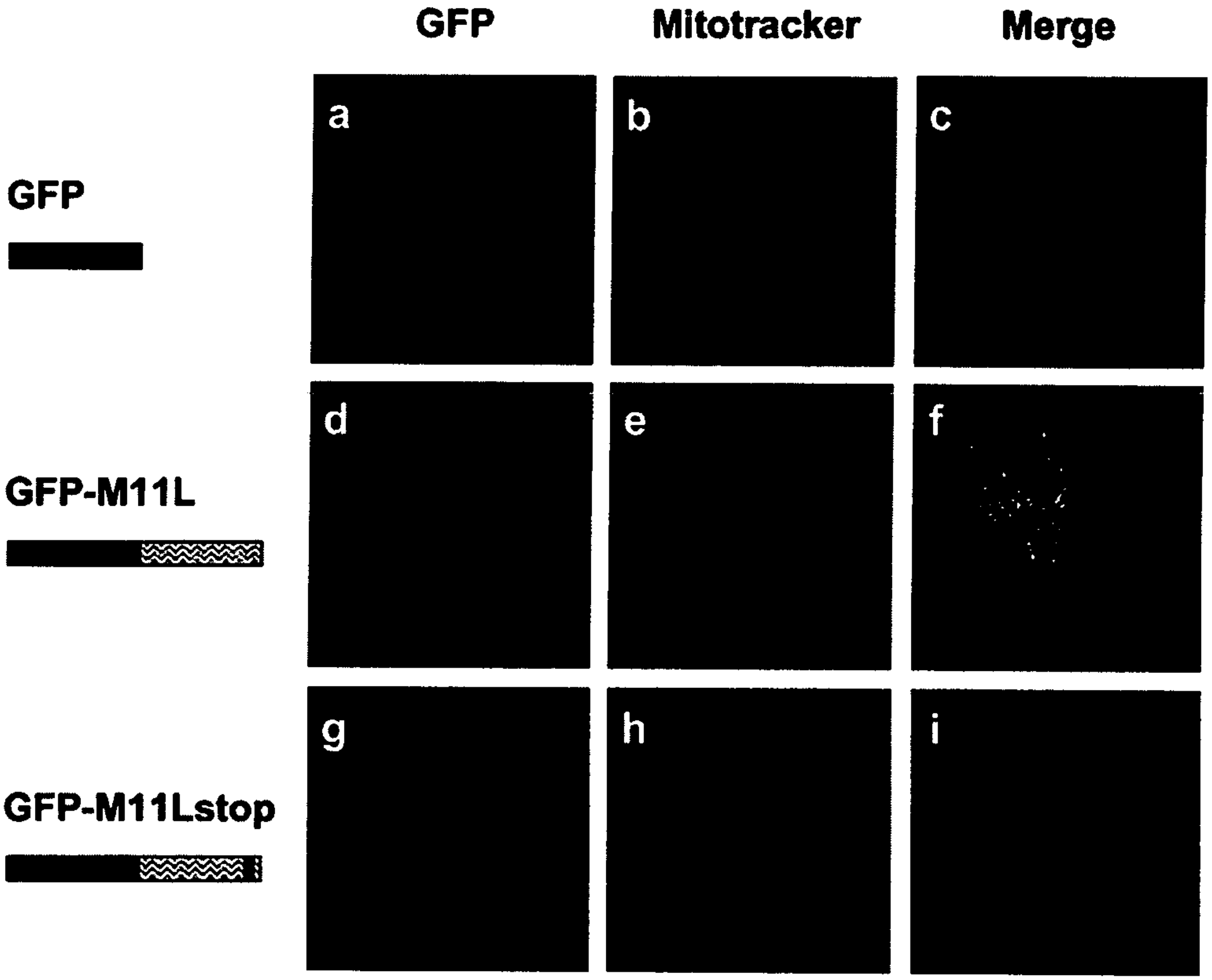

GFP
Mitotracker
Merge
GFP
a
b
c
GFP-M11L
d
e
f
GFP-M11Lstop
g
h
i

Figure 4

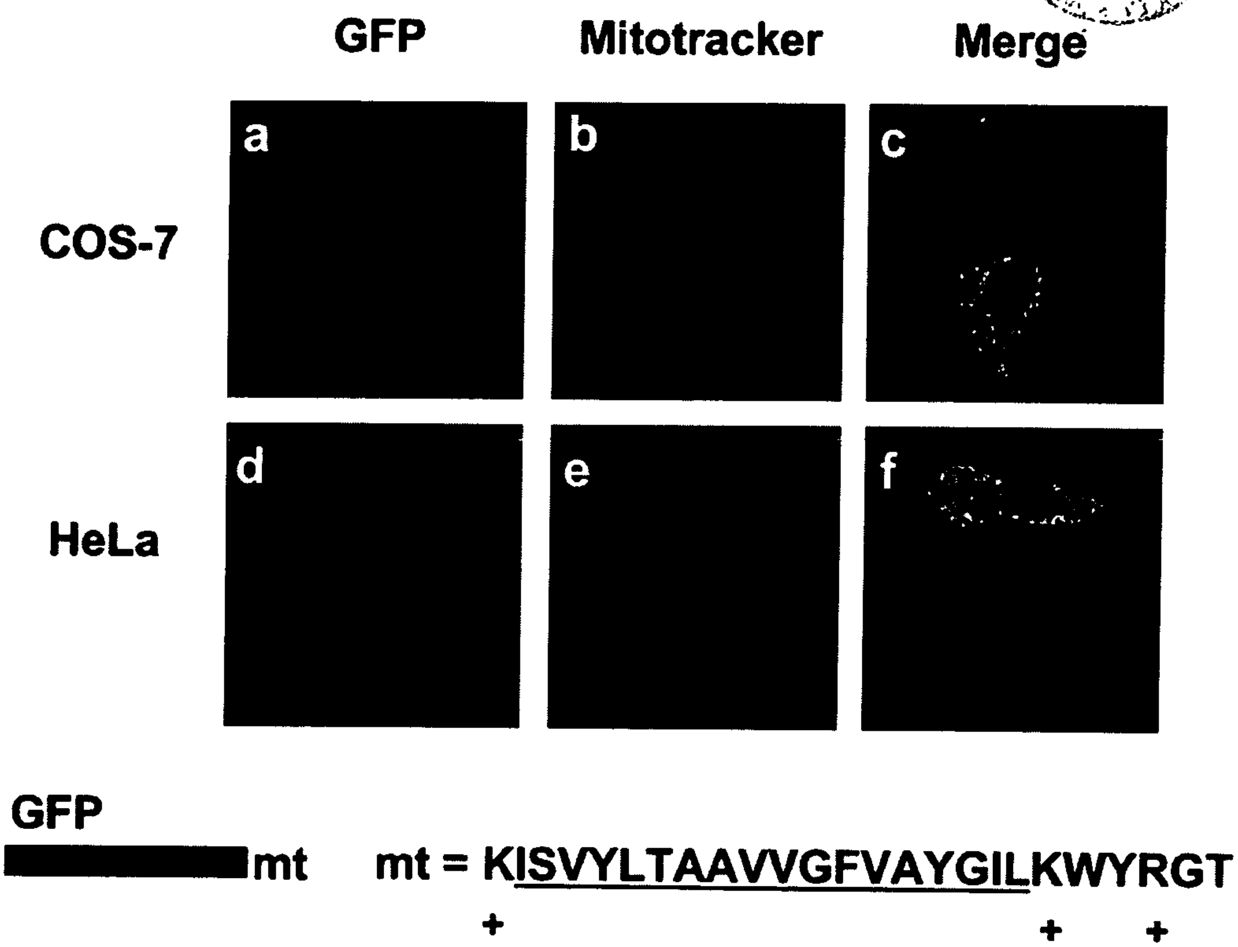

GFP
Mitotracker
Merge
COS-7
HeLa
a
b
c
d
e
f
GFP
mt
mt = KISVYLTAAVVGFVAYGILKWYRGT
+ + +

Figure 5

	positive charge	18-24 aa putative membrane-spanning domain	positive charge	positive tail	domain is a mitochondrial targeting signal	domain is required for function
M11L	K	ISVYLTAAVVGFVAYGIL	K	WYRGT	Y	Y
Bcl-2	K	TLLSLALVGACITLGAYLS	H	K	Y	Y/N
Bcl-X_L	R	WFLTGMTVAGVVLLGSLFS	R	K	Y	Y/N
Boo/Diva	R	LLIQAFLSGFFATAIFFIW	K	RL	?	?
CED-9	R	WSMIGAGVTAGAIGIVGVVVCG	R	MMFSLK	?	?
BHRF-1	R	FSWTLFLAGLTLSLLVICSYLFIS	R	GRE	Y	Y
KSbcl-2	R	MTALLGSIALLATILAAVAMS	R	R	?	?
Nip3	K	VFLPSLLLSHLLAIGLGIYIG	R	RLTTSTSTF	Y	Y
Nix	K	VFIPSLFLSHVLALGLGIYIG	K	RLSTPSA	Y	Y

Figure 6

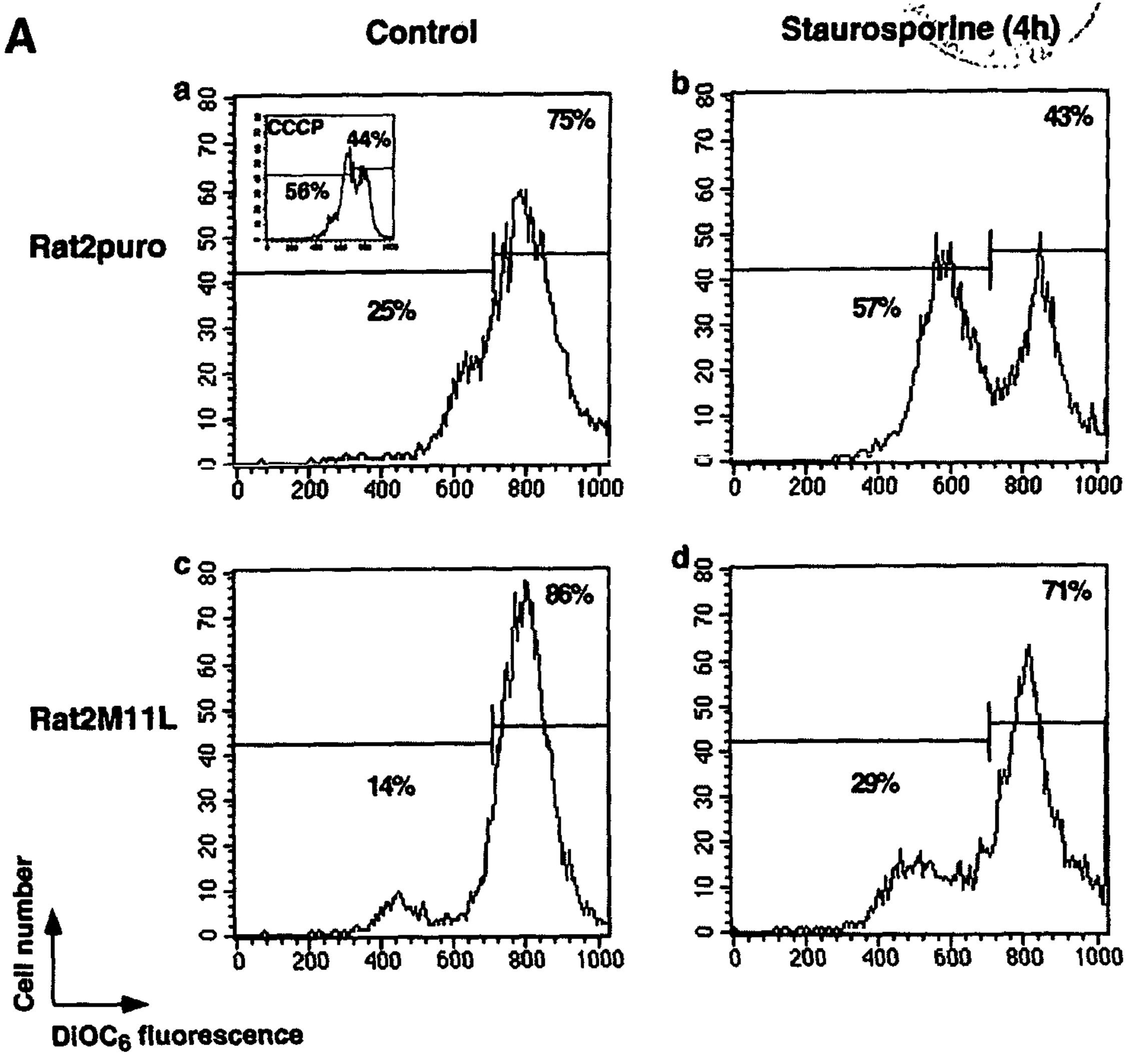

A
Control
Staurosporine (4h)
Rat2puro
Rat2M11L
a
CCCP
44%
56%
75%
25%
b
43%
57%
c
86%
14%
d
71%
29%
0
10
20
30
40
50
60
70
80
0
200
400
600
800
1000
Cell number
DiOC$_6$ fluorescence

Figure 6 (con't)

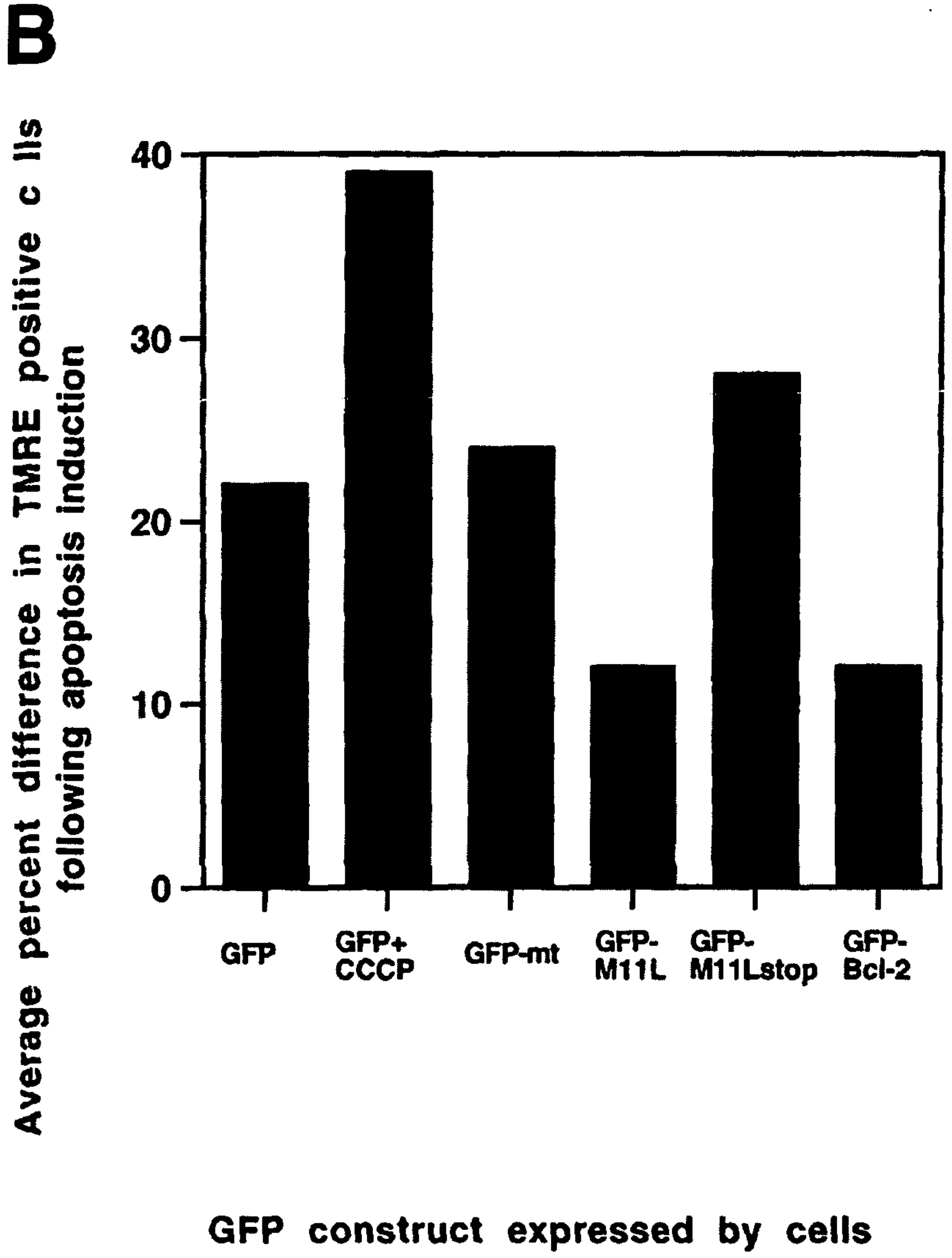
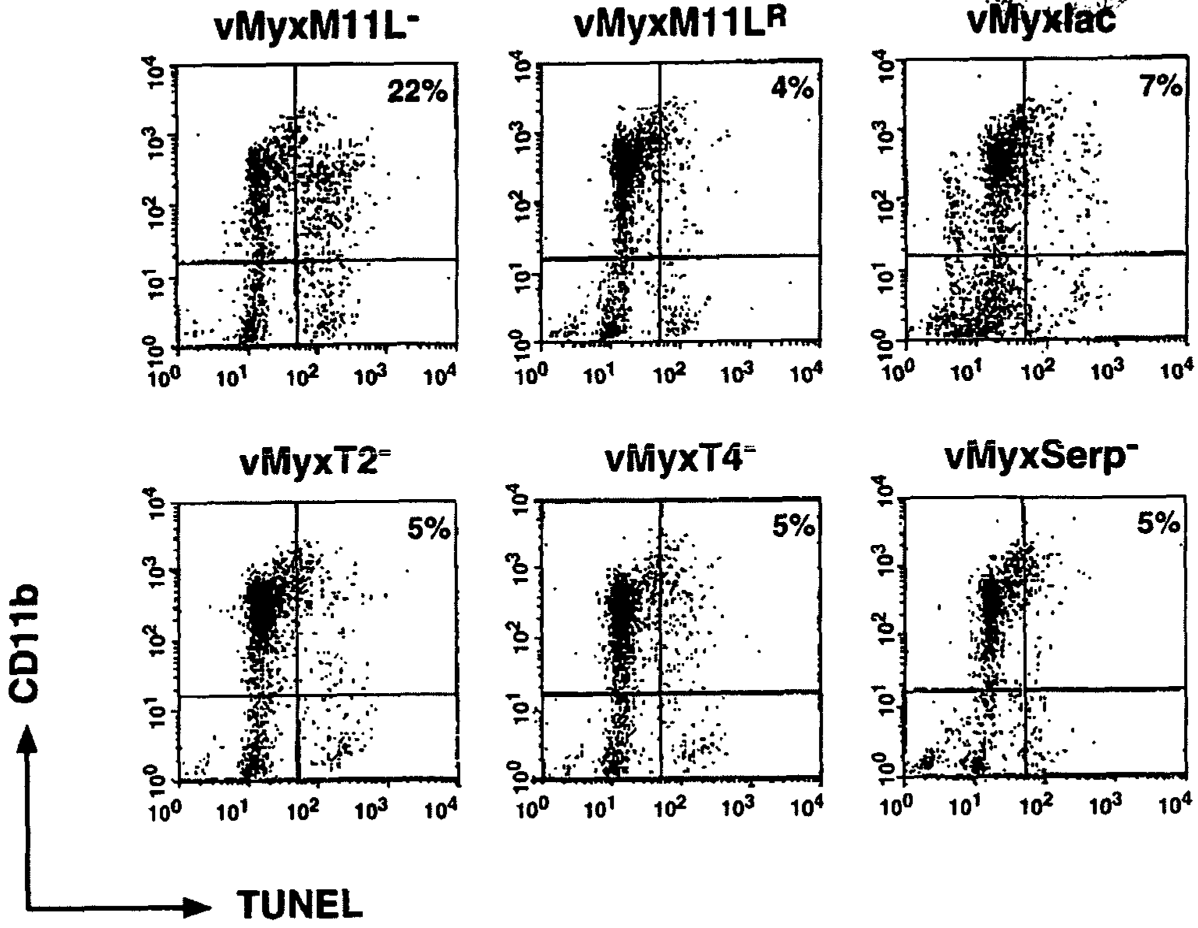


Figure 7


vMyxM11L⁻
22%
vMyxM11LR
4%
vMyxlac
7%
vMyxT2⁻
5%
vMyxT4⁻
5%
vMyxSerp⁻
5%
10⁰
10¹
10²
10³
10⁴
CD11b
TUNEL

Process to Identify M11L-interacting Proteins

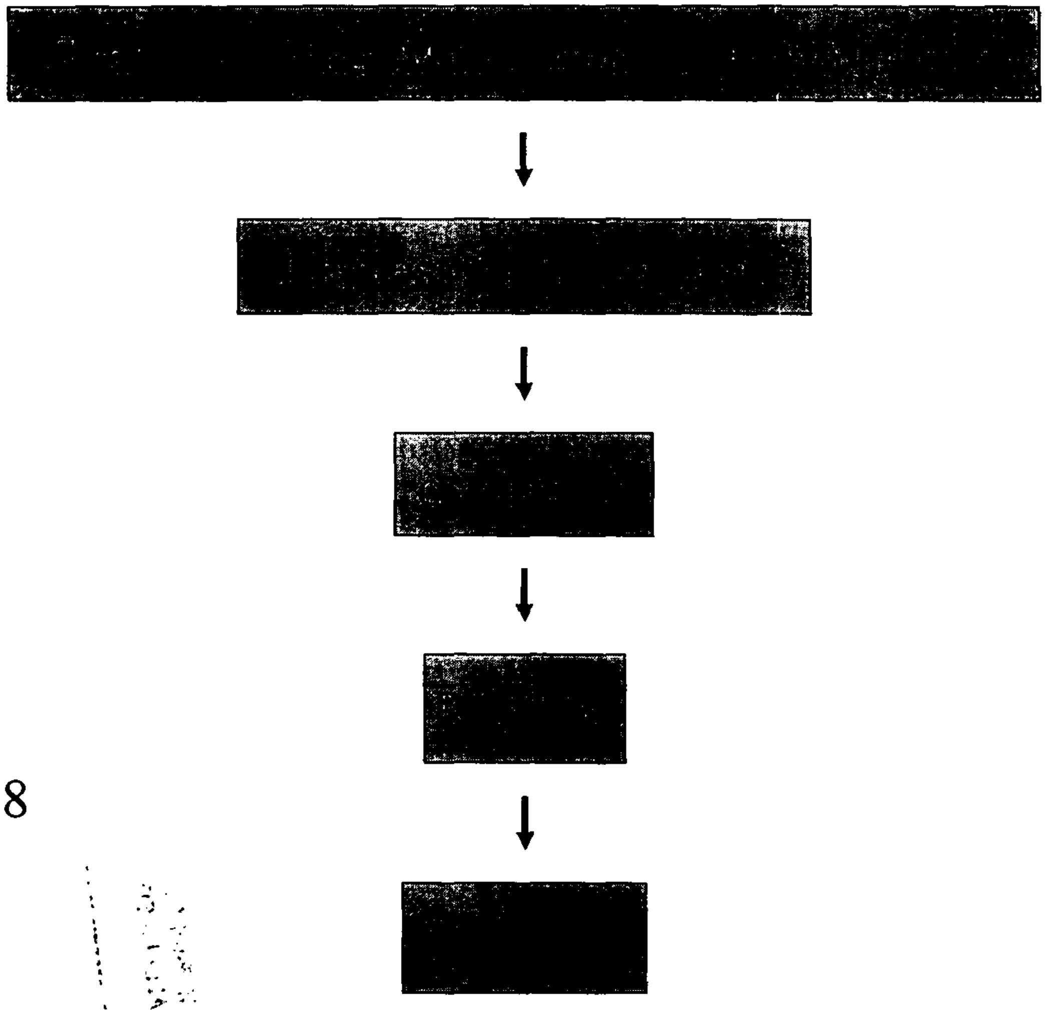


Figure 8

10090440.070902

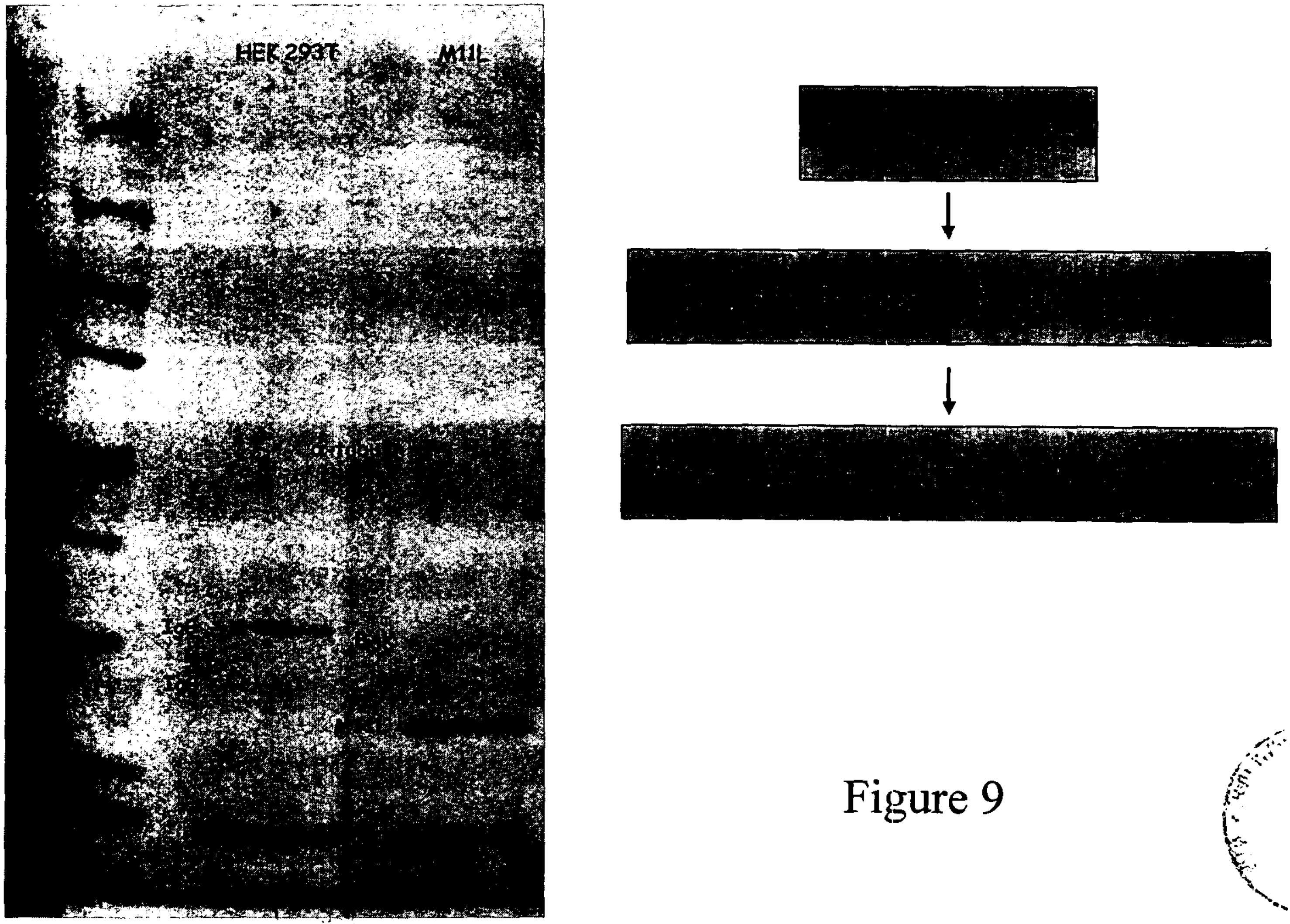

HEK 293T
M11L

Figure 9

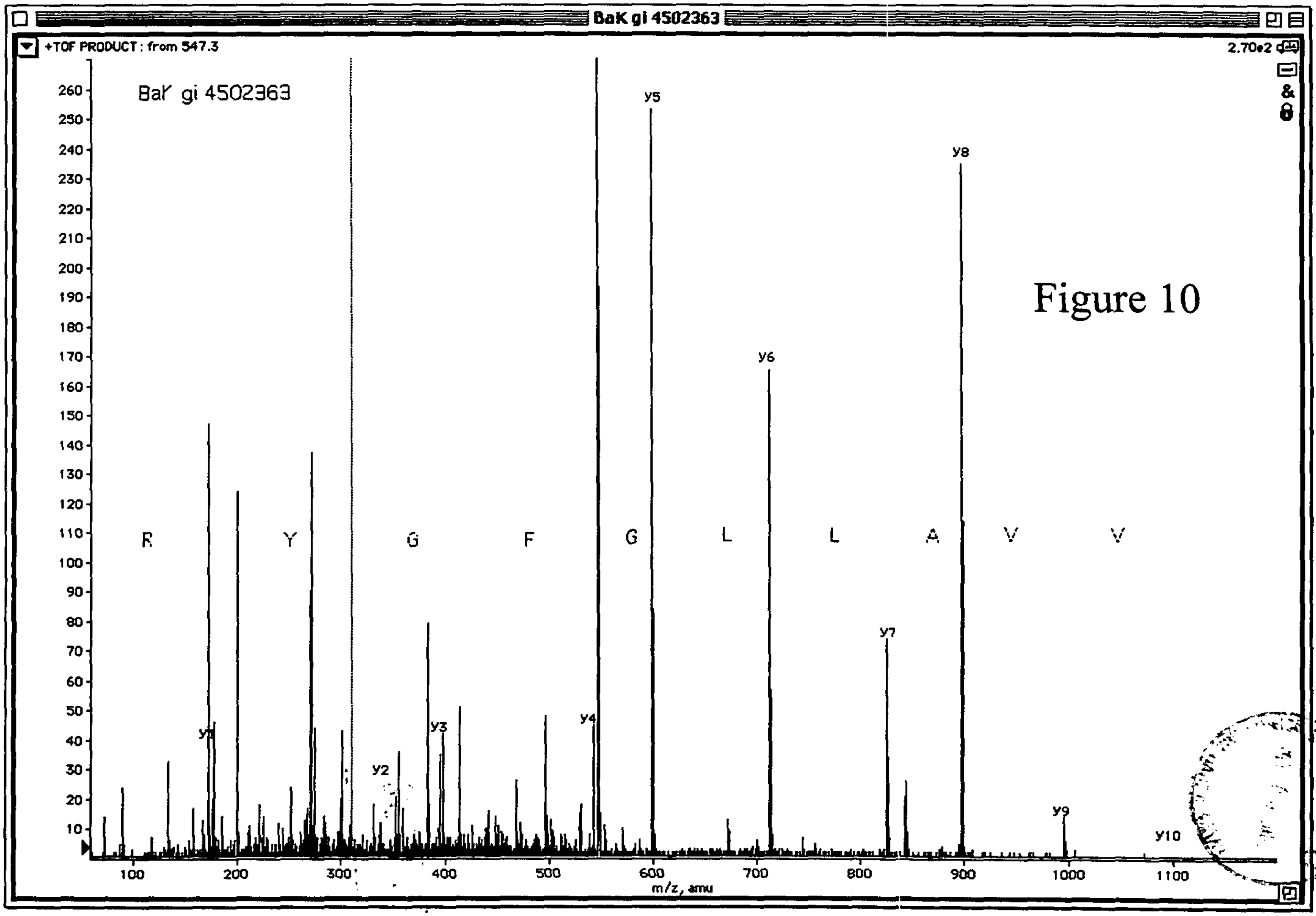

BaK gi 4502363
+TOF PRODUCT : from 547.3
2.70e2
Bak gi 4502363
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
R
Y
G
F
G
L
L
A
V
V
y1
y2
y3
y4
y5
y6
y7
y8
y9
y10
100
200
300
400
500
600
700
800
900
1000
1100
m/z, amu

Figure 10

Bak, a Bcl2 family member, was identified by Mass Spec

MASGQGPGPPRQECGEPALPSASEEQVAQD
TEEVFRSYVFYRHQQEQEAEGVAAPADPEM
VTLPLQPSSTMGQVGR**QLAIIGDDINR**RYD
SEFQTMLQHLQPTAENAYEYFTK**IATSLFE**
SGINWGRVVALLGFGYRLALHVYQH
GLTGFLGQVTRFVVDFMLHHCIARWIAQRGGWVA
ALNLGNGPILNVLVVLGVVLLGQFVVRRFFKS

Figure 11